PB-214 983

CHEMICAL FLOCCULATION OF MICROORGANISMS IN BIOLOGICAL WASTE TREATMENT

Mark W. Tenney, et al

Harvard University
Cambridge, Massaschusetts

October 1965

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 214 983

Journal

WATER POLLUTION CONTROL FEDERATION

CHEMICAL FLOCCULATION OF MICROORGANISMS IN BIOLOGICAL WASTE TREATMENT

Mark W. Tenney and Werner Stumm

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

OCTOBER 1965

CHEMICAL FLOCCULATION OF MICROORGANISMS IN BIOLOGICAL WASTE TREATMENT

Mark W. Tenney and Werner Stumm

Chemical treatment of wastes, despite early interest in such methods, fell into disfavor as improved biological methods were developed. It is widely recognized today that chemical treatment generally is not an acceptable alternative to biological methods in the processing of wastes. Biological treatment is far superior to chemical treatment for the removal of soluble organic matter. However, in recent years there has been a revived interest in special applications of chemical methods, especially for processing biologically treated effluents when additional purification is needed. Such third-stage chemical treatment has been proposed repeatedly, and now is in operation in some European wastewater treatment plants, for the removal of phosphates. Phosphate is one of the major factors contributing to progressive stream fertilization. The costs for third-stage chemical treatment are often quite high. Therefore, it appears worthwhile to investigate whether or not the additional costs resulting from such chemical treatment can be compensated for partially or fully by a reduction in the cost of biological treatment resulting from shortened detention times.

This research was undertaken to investigate to what extent chemical flocculation methods can complement, and partially substitute for, the biological treatment process.

Organic Removal and Biological Flocculation

In conventional activated sludge treatment the biological removal and conversion of organic constituents takes place in two phases. First, the organic waste is partially oxidized for free energy and partially synthesized into new microbial cells (substrate utilization phase). All parameters required for efficient biological substrate utilization are present in the first phase. In the second phase, which occurs in the same aeration tank, the flocculation of these newly synthesized cells is promoted (flocculation phase) (Figure 1A). Without flocculation of the microorganisms only the fraction of the organic matter which has been oxidized will be removed. Thus, the overall efficiency of a biological treatment process depends on flocculation.

The various factors that lead to bioflocculation are not very well understood yet. Operationally, at high concentrations of organic substrate (short aeration times) non-flocculant growth generally is observed, while after long aeration periods with very low concentrations of organic substrate conditions for excellent biological flocculation

Mark W. Tenney was, at the time this paper was prepared, Research Fellow, Department of Water Resources and Sanitary Engineering, Harvard University, Cambridge, Mass. He is presently Assistant Professor, Department of Civil Engineering, University of Notre Dame, Notre Dame, Ind. Werner Stumm is Professor of Applied Chemistry, Department of Water Resources and Sanitary Engineering, Harvard University, Cambridge, Mass.

The paper was presented at the 19th Annual Purdue Industrial Waste Conference, Purdue University, Lafayette, Ind., May 5–7, 1964.

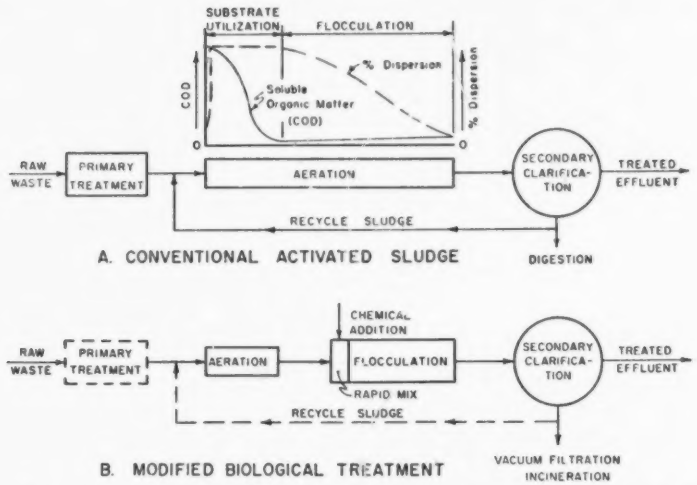

FIGURE 1.—Flow systems for conventional and modified biological treatment of wastewater, showing relationship to substrate utilization and bioflocculation phases.

predominate. The relative tendency for microorganisms to remain dispersed with time (length of aeration) is indicated by a dashed line representing percent dispersion in Figure 1A. Consequently, in conventional biological activated sludge treatment, long detention times and aeration periods are needed primarily for the promotion of flocculation.

The lower efficiency in organic carbon removal that is observed in high-rate activated sludge treatment is largely explainable by the fact that these effluents contain substantial amounts of suspended microorganisms. However, the requirements for most rapid substrate utilization, i.e., prolific growth of microorganisms, are incompatible with the requirements for optimum bioflocculation. The modifications of aerobic biological treatment that have been proposed represent attempts to compromise between the different requirements of the substrate utilization and the bioflocculation phases. At one end of the spectrum are the "dispersed-growth aeration" and "high-rate" activated sludge systems that exploit almost optimal substrate utilization rates, but fail in providing efficient separation of microorganisms from the solution. At the other end of the spectrum are the so-called "biosorption" and "extended aeration" processes, where optimum conditions for bioflocculation exist while unfavorable conditions for substrate utilization predominate, since operation is in the endogenous or declining growth phase.

Complementary Biological and Chemical Treatment

The incompatibility between the requirements for maximum substrate utilization and maximum bioflocculation raises the question of whether it is practical and economic to accomplish the objectives of both phases by biological means alone. While biological treatment is necessary for the elimination of soluble organic matter, chemical flocculation methods may be expected to agglomerate dispersed microorganisms and to remove suspended, colloidal, and non-dissimilated macromolecular material as efficiently as or even more efficiently than exclusive biological means. Since chemical treat-

ment can remove phosphate simultaneously, the effluents from such a complementary treatment process will promote neither heterotrophic activity (oxygen depletion) nor autotrophic activity (algal growth) in the receiving waters.

If chemical techniques are used for the flocculation of dispersed micoorganisms, the design of the biological stage, i.e., the substrate utilization phase, can be optimized without giving consideration to the bioflocculation stage. It is thus possible to reduce significantly the detention time of the biological stage and to operate this stage as a "high-rate, low-solids retention unit."

The Modified Process

A flow diagram for the proposed biological and chemical process is shown in Figure 1B. The waste, after conventional primary treatment, (it may be feasible and economically desirable to restrict primary treatment to comminution), enters an aeration tank of short detention time sufficient to remove soluble organic material from the liquid phase. The aeration tank effluent enters a rapid-mix chamber where the chemical flocculant is added, and then flows to a flocculation basin. From there the waste enters a settling tank, where the sludge and phosphate precipitates can be separated from the liquid phase. Because of the addition of a chemical flocculant the sludge will settle faster and, therefore, will require shorter detention times in the secondary clarifier. The settled sludge can be recycled or piped to additional treatment facilities.

The presence of flocculants in the excess sludge should facilitate considerably its vacuum filtration and dewatering. The settled sludge can be treated by anaerobic digestion. In anaerobic treatment, however, a large fraction (about 70–80 percent) of the phosphate that was incorporated in the aerobic microorganisms is released into the digester supernatant. Furthermore, if iron(III) is used as a phosphate precipitant, any phosphate bound to iron(III) will be released during the digestion because of reduction of the Fe(III) to Fe(II). In those instances where algal nutrients such as phosphate are of concern, digestion would be of value only if the digester effluent were not discharged to the stream or to the treatment plant.

The complementary chemical treatment proposed in this paper differs from a third-stage chemical treatment, in which fully biologically treated effluents are treated chemically with coagulants or absorbents, in the sense that it attempts to substitute chemical flocculation for bioflocculation. The cost of the chemical treatment would be compensated for by a reduction in cost of biological treatment resulting from shortened detention times.

A scheme illustrating the removal of organic matter by such a combination process is represented in Figure 2.

Mode of Bioflocculation

Considerable uncertainty exists about the mechanism of bioflocculation. However, consideration of the factors which influence the flocculative properties of microorganisms will lead to a greater understanding of the functioning of biological waste treatment systems and may in turn provide means by which the aggregation of microorganisms can be enhanced to achieve efficient phase separation in biological treatment.

Bacteria as Biocolloids

At one time it was thought that special floc-forming bacteria (e.g., *Zoogloea ramigera*) were primarily responsible for producing activated sludge floc. Protozoa also were thought to cause flocculation by excreting a gelatinous slime to trap bacteria before ingestion. However, it has been realized that most microorganisms can, under certain circumstances, form ag-

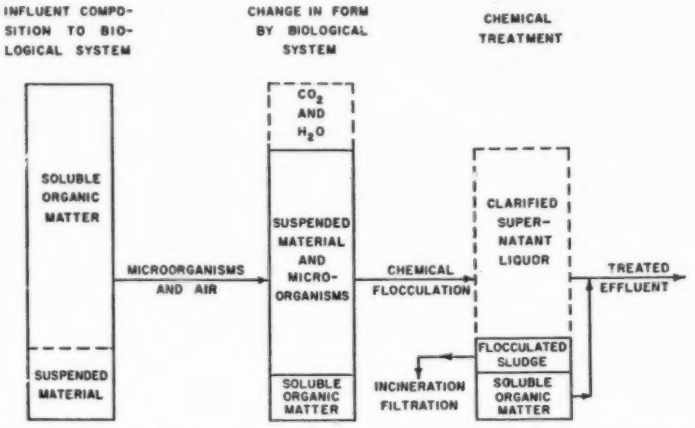

FIGURE 2.—Scheme for removal of organic material by a combination of biological and chemical treatment.

gregates (1) (2) (3). Bacteria and other microorganisms such as viruses, algae, and protozoa, can be considered to be hydrophilic biocolloids. They carry a net negative charge within the pH-range of interest. The stability of a dispersion of hydrophilic biocolloids depends on the forces interacting between the particles themselves as well as on the forces interacting between the particles and the water. Hence, electrostatic repulsive forces do not explain fully the stability of bacterial suspensions. Bacterial suspensions can form stable dispersions even at the isoelectric point. Thus, the concept of the zeta potential (i.e., opposing effects of repulsive interactions between the electrical double layers and of attraction by Van der Waal's forces) alone cannot account satisfactorily for the phenomenon of bioflocculation. The interaction of the hydrophilic surface with the solvent (water) also is an important stability factor.

Colloid-chemical investigations on the flocculation of bacteria are made difficult by the great variability of the bacterial surface composition. Proteins, lipids, polysaccharides, and nucleic acids, combined in various proportions, occur on the surface in the form of cell walls, capsules, flagellae, fibrils, filaments, and limbriae of various kinds. The inhomogeneity of the microbial populations encountered in waste treatment systems represents additional difficulties for a simple interpretation of observed facts.

The external surface of bacteria essentially consists of amphoteric and hydrophilic groups. It acquires its charge through acid-base interactions of the functional ionogenic groups. Thus, the surface charge density of bacteria is strongly pH-dependent. Adsorption of simple ions to the surface is limited by its hydration and is less important in establishing the surface charge density. The isoelectric points of bacteria are at very low pH values (pH 2–4) and are significantly lower than those of proteins (pH 5–8). This suggests the occurrence on the external surface of substances that have lower isoelectric points than proteins, such as glutamic acid, lipids, and gluco- and mucopolysaccharides.

Factors Affecting the Stability of Microbial Dispersions

The stability of a bacterial suspension depends on the presence of surface-modifying agents in the medium. Hydrogen ion affects the charge char-

acteristics and influences the stability, at least to a certain degree. The stability of a bacterial dispersion also depends on the physiological conditions of the cells. Microorganisms in aerobic waste treatment plants generally remain dispersed as long as they grow prolifically. Conditions for bioflocculation improve in the declining growth phase and become optimal in the endogenous phase, especially after extensive aeration (Figures 1A and 12D). It appears that the properties of the external surface of the microorganisms are changed when their physiological conditions alter in going from active growth to endogenous respiration.

Although changes in charge density characteristics of bacteria with the age of the culture have been observed in individual cases (4) (5) (6) (7) (8), it is doubtful that agglomeration of microorganisms is brought about simply by reduction in charge density and by a decrease in electrostatic repulsion. Charge reduction is not necessarily a prerequisite for bioflocculation (3) (9). In fact, negative colloidal dispersions have been flocculated effectively by negatively charged polyelectrolytes (10) (11) (12).

Proposed Mechanism for Flocculation

Polymers of biological origin, especially polymers which carry electrical charges, are of great importance in the organization of biological structure. Proteins, protamines, nucleic, pectic, alginic acids and numerous polyacids of bacterial origin are natural polyelectrolytes. Such natural polyelectrolytes are of significance in the interaction of protamines and histones with nucleic acids in the chromosomes and in the agglutination of red blood cells (13) (14). Thus, it appears plausible to interpret bioflocculation in terms of polyelectrolyte interaction. Natural polymers such as complex polysaccharides and polyamino acids are excreted or exposed at the surface predominantly during the declining-growth and the endogenous-respiration phases. It is possible that such polymeric substances are excreted under all physiological conditions, but that under conditions of prolific growth, new surfaces are created faster than surfaces can become covered with such polymers. These polymeric molecules are of sufficient length to form bridges between microbial particles.

In accordance with such a hypothesis, polyacids of bacterial origin have been used successfully to flocculate bacteria. For example, suspensions of *E. coli* and *M. aureus* are flocculated readily by microquantities of polylysine (13). Similar phenomena have been observed with human erythrocytes (15). It has been well established that sewage bacteria, in the endogenous phase, accumulate substantial amounts of polysaccharides (3) (16) (17) (18). Such material represents a large fraction of the residual organic matter (COD) in extended-aeration-type units (19) (20) (21). We have observed that centrifugation of bacteria which have been maintained under endogenous conditions can shear off substantial quantities of loosely bound capsular material.

Experimental Procedures

Culture Units

Flocculation experiments were carried out with samples from batch cultures and from effluents of "continuous culture" (so-called "complete mixing" activated sludge) systems. The tank of the continuous culture unit had a volume of 2 l, was well aerated, and was fed continuously, by means of a positive displacement pump, with an organic medium that contained in tap water 2.3 g/l nutrient broth, 1.0 g/l glucose, and 0.075 g/l sodium oleate. Chemical treatment of this medium alone, i.e., without prior biological mediation, did not remove organic material to any extent (Figure 3). An 1,800-ml portion of the organic

medium containing a COD-equivalent of 4.44 g/l was passed through the reactor each day. The theoretical dilution rate was 0.9/day. Three such units were run in parallel.

During steady-state and continuous operation, the microorganisms in these treatment units grew under the same environmental conditions. All the cells in the unit and the effluent, therefore, should have been in the same physiological state; in the effluent their concentration and that of the residual substrate should have been constant at all times. Such constancy of the effluent composition is necessary in order to evaluate the various factors involved in chemical flocculation.

For the batch experiments, a medium made up of 2.0 g/l glucose, 0.625 g/l $(NH_4)_2SO_4$, and 0.160 g/l K_2HPO_4 in tap water was used in a mechanically stirred and well-aerated cylinder having a volume of 32 l.

Flocculation Experiments

Proper consideration of the effects of pH on the destabilization of microbial colloids and on the extent of ionization of polyelectrolytes, hydrolysis of multivalent metal ions, and chemical precipitation reactions (phosphate precipitation) can be secured only if pH is kept rigorously constant during any one flocculation experiment. The method which has proved most useful in this laboratory employs an automatic titrator (22) for the external control of this parameter. The method used in these experiments is as follows: A 200-ml sample in a 400-ml beaker is brought to the desired pH by addition of appropriate chemicals (HNO_3 or NaOH). The automatic titrator, the glass electrode of which is immersed in the coagulation jar, is set to the preselected pH. While the desired quantity of flocculent chemical is added slowly to the solution under constant stirring, the automatic titrator doses automatically and continuously into the test solution the quantity of base (NaOH or $NaHCO_3$) necessary to maintain constant pH and alkalinity throughout each flocculation experiment. Such a procedure is especially necessary if polyvalent metal ions are used as flocculants; any hydroxide ion consumed in the metal-ion hydrolysis reaction is replaced automatically by OH^- of the titrator base essentially as quickly as

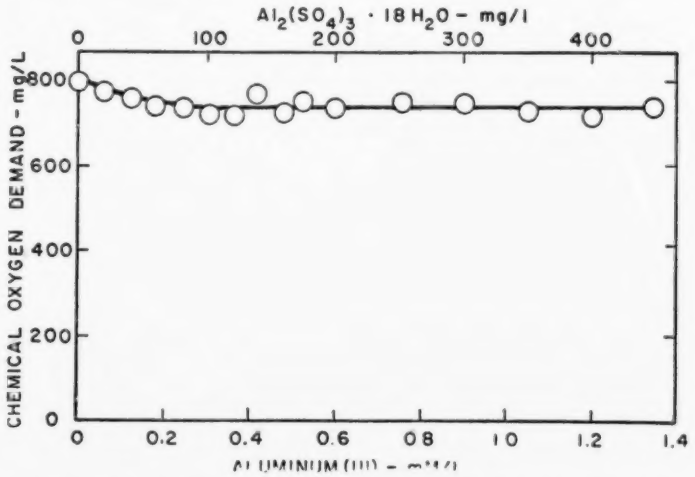

FIGURE 3.—Effect of flocculant dose on COD of waste not treated biologically before flocculation.

the hydrolysis proceeds. After an additional slow stirring at 30 rpm for 15 min, the sample was settled in a 250-ml graduated cylinder for 1 hr. The supernatant then was analyzed. Data obtained from a series of such flocculation experiments have been presented as flocculation curves by plotting of parameters such as residual COD, fraction of cells remaining dispersed, or remaining phosphate against chemical dosage. Each curve is valid for one pH only.

Analytical Methods

Determinations of dichromate consumption were made by using a procedure recommended for small samples (23); the results are expressed in terms of COD. Glucose was determined by an enzymatic method using glucostat procedure.* Phosphate was analyzed by use of $SnCl_2$ (24). Estimations of bacterial numbers were made from counts obtained on spot plates of Tryptone Glucose Extract Agar. In the growth experiments concentration of microorganisms, as measured by dry weight of suspended solids, was estimated after filtration with membrane filters (25). The experiments were conducted in laboratories with a controlled temperature of 21° ± 2°C.

COD measurement by dichromate consumption was selected as the principal test to determine the extent of flocculation of microorganisms. Since the samples contained little suspended material other than microorganisms, the removal of organic matter by flocculation can be accounted for almost completely by the removal of microorganisms. The equivalent COD of the microorganisms was determined by the difference between an unfiltered sample and a membrane-filtered sample. The relationship may be expressed as: $COD_{cells} = COD_{total} - COD_{soluble}$. The relative degree of flocculation of a system was evaluated in terms of percent dispersion, the ratio of the COD contribution of the microorganisms remaining suspended after 1 hr of settling to the COD contribution of all the microorganisms:

$$\text{Percent dispersion} = \frac{COD_{1\text{-hr settling}} - COD_{soluble}}{COD_{total} - COD_{soluble}} \times 100$$

Results and Discussion

The interpretation of chemical treatment for the flocculation of microorganisms, ideally, should be guided by considerations which apply to natural bioflocculation. In the expectation that nature will be simulated, chemical materials added for the flocculation of dispersed microorganisms should fulfill the following requirements: (*a*) they should become strongly bonded to the microbial surface by chemical or electrostatic forces; and (*b*) these materials should be able to form linear polymers that are capable of bridging and uniting the microbial particles of a dispersion into a loose three-dimensional structure. The results which we have obtained on the flocculation of microorganisms with inorganic and synthetic organic chemicals are interpreted with the aid of the postulated polymer-bridge model.

Flocculation Results

Most experimental data have been obtained from an interpretation and evaluation of flocculation curves constructed by plotting dosages of chemical flocculant as abscissa and a suitable variable, such as concentration of organisms remaining dispersed, as ordinate. Each flocculation curve is constructed from results obtained in a series of flocculation experiments, each carried out under the same controlled conditions of pH, degree of agitation, temperature, etc. Figure 4 gives a schematic representation of a typical flocculation curve obtained for the chemical treatment of a biologically

* Developed by Worthington Biochemical Corp.

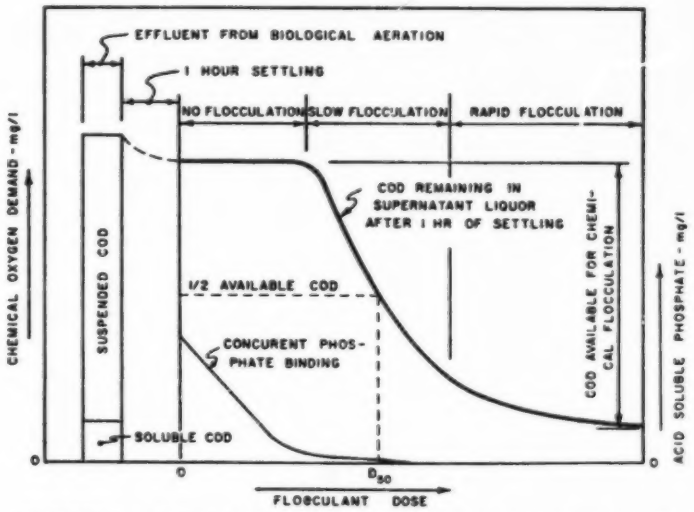

FIGURE 4.—Schematic flocculation curve for the removal of organic matter and phosphate by Al(III) at constant pH.

"pre-treated" waste in which the microorganisms are dispersed.

Flocculation curves frequently can be characterized expediently by the D_{50} parameter (Figure 4), i.e., that dose of flocculent which is required to reduce the concentration of dispersed or dissolved material by 50 percent. This D_{50} dose is generally reproducible and can be determined with good accuracy as approximately the largest slope in the flocculation curve. The D_{50} parameter has been used in establishing the quantitative relationship between concentration of dispersed cells or phosphate and the dose necessary for their flocculation or precipitation.

The results given in Figures 5 to 11 refer to effluents of the continuously operated bench-scale biological treatment units. Results depicted in Figure 12 refer to batch experiments, in which the microorganisms in the various samples investigated are under different physiological conditions varying with the growth cycle.

Synthetic Polyelectrolytes

A large number of commercially available polyelectrolytes were tested with effluent from the continuous-culture units. Under conditions in this test, only cationic polyelectrolytes were able to flocculate microorganisms efficiently. This is in accord with the results of other investigations (13) (26) (27). While in the interaction of polyelectrolytes with clays, SiO_2, or hydrous metal oxides, specific chemical short-range forces involved in adsorption generally outweigh small long-range electrostatic forces (12), it appears that in the interaction of polyelectrolytes with cells, electrostatic forces are necessary in order to "bond" positively charged segments of the polyelectrolyte to the microbial surface (13) (28). If bridging between the cells is to be accomplished, the polymer must extend from the surface so that it is capable of being adsorbed to the "bare" surfaces of other approaching cells, or of interacting with extended segments of other similarly adsorbed polymers.

Figure 5 gives a typical flocculation curve showing residual dispersed microorganisms vs. concentration of added polyelectrolyte at constant pH. Hydrogen ion can affect the floccula-

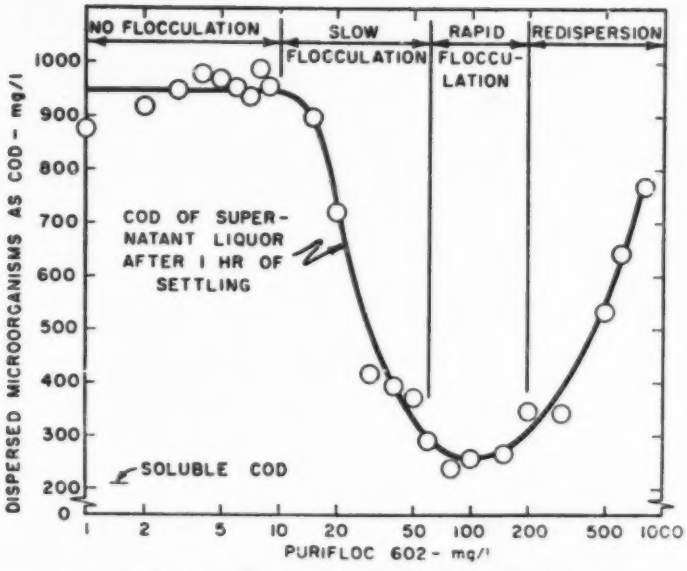

FIGURE 5.—Effect of polyelectrolyte dose on removal of microorganisms.

tion in various ways: it influences the charge characteristics of both bacteria and polyelectrolyte, and it determines the extent of coiling and influences the diffusion characteristics of the polymer. Factors that influence the degree of flocculation for a given polyelectrolyte include pH, extent of agitation (Figure 6), temperature, and the ionic composition of the solution.

Stoichiometry:—Values of the amount of polymer necessary to flocculate 50 percent of the organisms under controlled conditions of pH, agi-

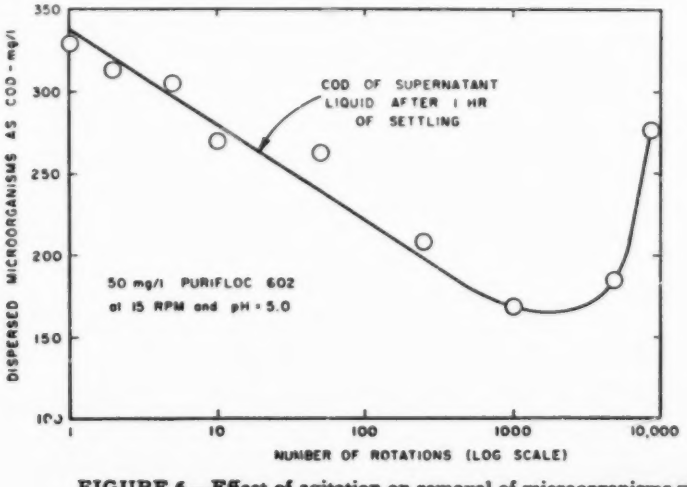
DISPERSED MICROORGANISMS AS COD - mg/l
COD OF SUPERNATANT LIQUID AFTER 1 HR OF SETTLING
50 mg/l PURIFLOC 602
at 15 RPM and pH = 5.0
NUMBER OF ROTATIONS (LOG SCALE)

FIGURE 6.—Effect of agitation on removal of microorganisms with cationic polyelectrolyte.

tation, and temperature are presented in Figure 7. A linear relationship between the amount of polymer and the number of microorganisms, i.e., total specific surface area expressed, is obtained. Qualitatively, this "stoichiometric" relationship can be explained by assuming that a certain and approximately constant fraction of the surface of the cells has to become covered with extended polymer segments.

Presumably, almost all polymer molecules that have been added to the solution become adsorbed at the microbial surfaces. It appears from our results that the amount of polyelectrolyte required to cause flocculation is of the order of 20 μg/mg of cell (dry weight). This amount appears to be only a fraction of that which can ultimately be adsorbed at the cell surface. The addition of an excess of polyelectrolyte to the bacterial suspension thus leads to a partial redispersion of the organisms (Figure 5). As soon as too large a fraction of the cell surface becomes covered with polyelectrolyte, the surfaces which are now positively charged start to repel each other. The results given in Figure 6 partially support such an interpretation. With a given microorganism-polyelectrolyte suspension, the extent of polyelectrolyte adsorption apparently increases with prolonged agitation. Hence, optimum flocculation is obtained only for a certain time span and if the suspension is agitated for longer time periods it redisperses, presumably because of more extensive adsorption of polymer segments on the cell surface.

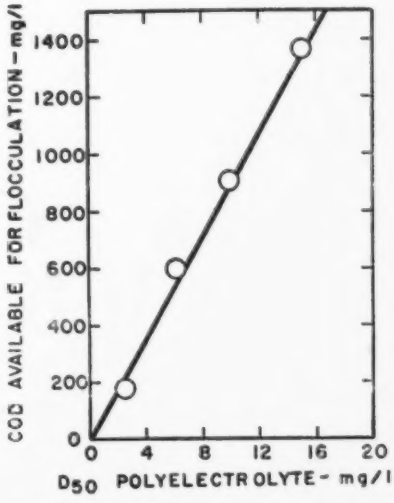

FIGURE 7.—Relationship of initial concentration of microorganisms to dose of cationic polyelectrolyte required to reduce original concentration by 50 percent.

Multivalent Metal Ions

Typical metal coagulants, such as aluminum(III) and iron(III), are known to be efficient conditioners of sludge. In the flocculation of microorganisms by metal coagulants, phenomena similar to those encountered in flocculation with polyelectrolytes are observed. These include: (*a*) a strong pH-dependence on the efficiency of flocculation (Figure 8); (*b*) a stoichiometric relationship between the concentration of organisms and the metal ion dose necessary for the flocculation (Figure 9); and (*c*) a very similar shape of the flocculation curve (Figure 10).

The pH-dependence of flocculation by metal coagulants is instructive (Figure 8). The optimum pH range for effective flocculation is around pH 5 to 6 for aluminum(III) (Figure 8A), and around pH 4.5 to 5.5 for iron(III) (Figure 8B). Within this pH range, all the aluminum(III) and iron(III) ions undergo rapid hydrolytic reactions and form hydroxopolymers (22). At pH values lower than this optimum range, where the free metal ions Al^{+3} and Fe^{+3} are prevalent, and in the neutral and alkaline pH range, where hydrolysis leads immediately to hydrous metal oxides, ready flocculation of microorganisms does not occur. In the acid pH range ($pH < 4$) where the charge of the

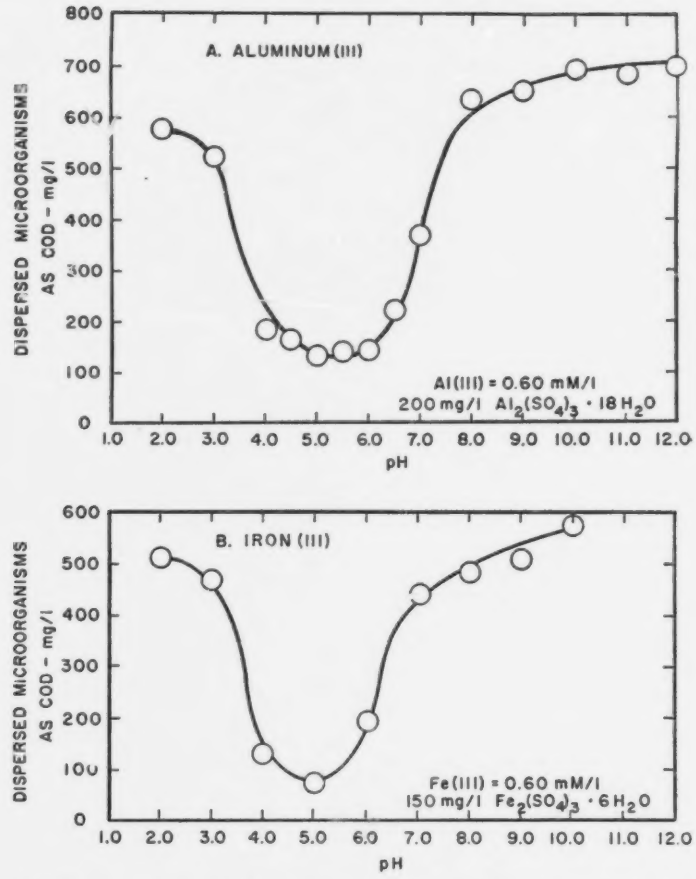

FIGURE 8.—Effect of pH on flocculation of dispersed microorganisms by Al(III) and Fe(III).

microbial surface is less negative than in nearly neutral solutions and thus supposedly more amenable to coagulation, free multivalent ions of iron(III) and aluminum(III) at substantial concentrations, far above the critical concentration necessary for the coagulation of lyophobic colloids, are not capable of coagulating microbial cells.

Hydrolysis products alone, and not the free metal cations or hydrous metal oxides, can produce agglomeration of microorganisms. This suggests that the aggregation produced by aluminum (III) or iron(III) salts is caused by the interaction of linear polymers, resulting from the hydrolysis of these salts, with the dispersed cells. The metal ions at the end of these short-chain polymers will be bonded predominantly by chemical forces onto the ionogenic groups of the microbial surface.

Flocculant Dose and Cell Concentration:—As in the case of polyelectrolytes, a certain minimal fraction of the surface must become covered with metal-ion flocculant before agglomeration can take place. Here again, at a given pH, a "stoichiometric" relationship between flocculent dose and concentration of dispersed cells is well

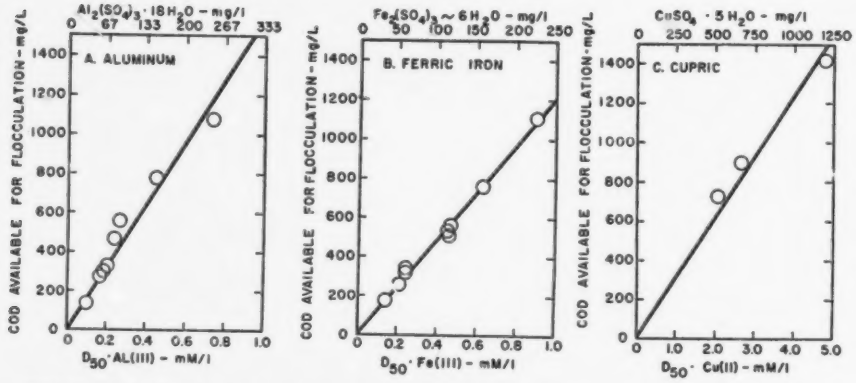
A. ALUMINUM
B. FERRIC IRON
C. CUPRIC
COD AVAILABLE FOR FLOCCULATION - mg/L
$Al_2(SO_4)_3 \cdot 18H_2O$ - mg/l
$Fe_2(SO_4)_3 \sim 6H_2O$ - mg/l
$CuSO_4 \cdot 5H_2O$ - mg/l
D_{50} · AL(III) - mM/l
D_{50} · Fe(III) - mM/l
D_{50} · Cu(II) - mM/l

FIGURE 9.—Relationship of initial concentration of microorganisms to dose of metal ion required to reduce initial concentration by 50 percent.

established (Figure 9). As described under "Experimental Procedures," in each experiment pH was controlled externally with the aid of an automatic titrator. Without such control, the pH would progressively decrease with increase in flocculant dosage and the results then no longer would be indicative of a stoichiometric relationship between dosage and concentration of microorganisms. The flocculant dose requirements are approximately equivalent for iron(III) and Al(III). Much larger equivalent doses are necessary for hydrolyzable bivalent metal ions such as Cu(II). Nonhydrolyzable cations such as Ca^{+2} do not aid substantially in the flocculation.

Settling of Flocculated Suspensions

Further support for the hypothesis that the agglomeration of microorganisms is brought about by the action of hydrolyzed metal ions or synthetic

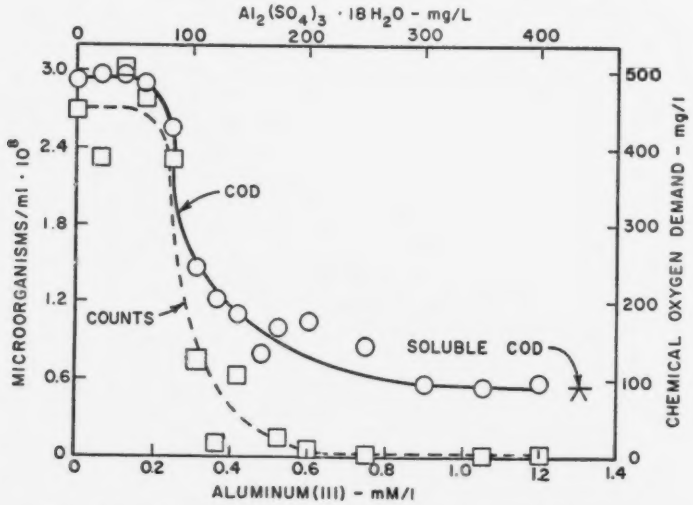

FIGURE 10.—Effect of flocculant dose on concentration of microorganisms in effluent of biological treatment process.

polyelectrolytes, both of which act as linear polymers uniting the cells of the dispersion into a loose three-dimensional structure, is lent by observations on the settling behavior of flocculated suspensions. In these flocculated dispersions settling occurs by subsidence rather than by sedimentation. The distinction between these two modes of settling and their relation to colloid chemistry have been delineated by La Mer and Healy (12). According to these authors, in a coagulated colloidal dispersion the individual particles sediment as independent units obeying as a limiting law the law of Stokes. On the other hand, in a flocculated dispersion, as soon as the bridging becomes effective, the entire solid content subsides by a uniform compression of the flocs.

In dispersions of bacteria that have been flocculated with natural polyelectrolytes (bioflocculation) and synthetic polyelectrolytes, or with hydrolysis products of polyvalent metal ions, the settling behavior is characterized by subsidence. In most cases the progress of settling can be followed by observing the height of a sharp subsidence line as a function of time.

Chemical Removal of Phosphate

The investigations of various authors (29) (30) (31) have demonstrated that the continuous removal of phosphate from wastewater treatment plant effluents by precipitation with Fe(III) and Al(III) or Ca(II) does not pose any technical difficulties. Previous investigations in the authors' laboratory (32) have shown that phosphate removal by polyvalent metal ions occurs by precipitation rather than by coagulation or adsorption.

pH-Dependence and Stoichiometry:—Under favorable pH conditions the quantity of metal ion needed for the phosphate precipitation can be estimated from the stoichiometry of the reaction:

$$Al(III) + H_nPO_4^{-(3-n)} = AlPO_4(s) + nH^+$$

i.e., one mole of Al(III) is necessary to precipitate one mole of phosphorus. Under practical conditions, results can be obtained which are in reasonable accord with this theoretical stoichiometry if the pH of the waste is properly adjusted (Figure 11A).

It has been shown earlier (22) (32) (33) that the pH-dependence of the metal ion-phosphate interaction can be explained in a quantitative way by considering the solubility product of the metal phosphate and the hydrolysis and acid-base equilibria of the metal and phosphate ions, respectively. The pH of optimum precipitation decreases with increasing phosphorus concentration in solution.

Al^{+3} has a stronger affinity for phosphorus than for the ionogenic groups of the microbial surface. Therefore, if Al(III) is added to a suspension of microorganisms containing phosphate, it will react first with the latter and will become a flocculant for the microorganisms only after the majority of the phosphate has been precipitated. Such a reaction sequence is depicted in Figure 4 and explains, at least in part, the initial zone where no flocculation of microorganisms occurs.

Colloid-Chemical Properties of $AlPO_4$:—Precipitates of $AlPO_4$ and $FePO_4$ have a strong tendency to form stable colloidal dispersions (34), except in the neighborhood of the isoionic point. Coarse and settleable precipitates are obtained at the pH of optimum precipitation only if the concentration of added Al(III) is very nearly that of the initial phosphorus concentration. As long as [Al(III)] < [P], negatively charged $AlPO_4$-colloids, which do not settle readily, will be formed. Such colloids are removable by membrane filtration, however. The colloid-chemical behavior of $AlPO_4$ precipitates provides a ready explana-

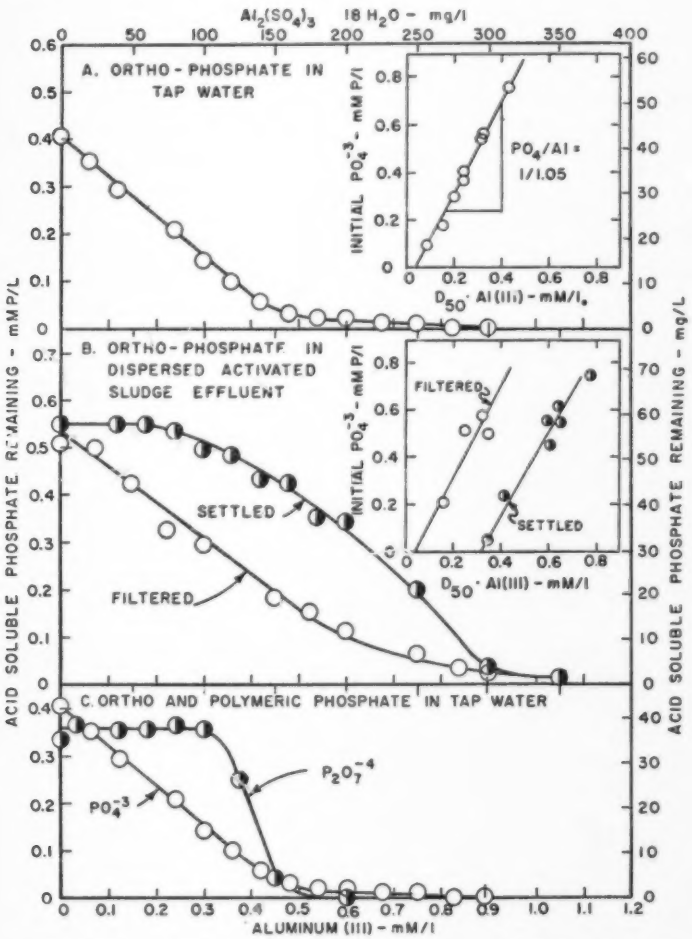
$Al_2(SO_4)_3$ 18 H_2O - mg/l
A. ORTHO-PHOSPHATE IN TAP WATER
INITIAL PO_4^{-3} - mM P/l
PO_4/Al = 1/1.05
D_{50} Al(III) - mM/l
B. ORTHO-PHOSPHATE IN DISPERSED ACTIVATED SLUDGE EFFLUENT
FILTERED
SETTLED
C. ORTHO AND POLYMERIC PHOSPHATE IN TAP WATER
$P_2O_7^{-4}$
PO_4^{-3}
ACID SOLUBLE PHOSPHATE REMAINING - mM P/L
ACID SOLUBLE PHOSPHATE REMAINING - mg/L
ALUMINUM (III) - mM/l

FIGURE 11.—Effect of flocculant dose on removal of phosphorus at pH 6.

tion for the different shape of the precipitation curves obtained depending on whether residual [P] is determined in filtered samples or in the supernatant of sedimented samples (Figure 11B).

Polymeric Phosphate: — Municipal wastewater contains, in addition to orthophosphate, the polymeric phosphates, pyrophosphate, metaphosphate, and tripolyphosphate. These polyphosphates also can be precipitated by Al(III) with a stoichiometric relationship very close to Al : P = 1. However, soluble polyphosphato-aluminum complexes are formed if the equivalent concentration of phosphorus exceeds the molar concentration of Al(III); thus, no precipitates are obtained until the concentration of added Al is equal to or slightly greater than the initial P concentration (Figure 11C).

Simultaneous Removal of Phosphate and Microorganisms:—Al(III) can be used for the removal of both phosphates and microorganisms. A considerable degree of control over the efficiency of removal can be achieved

by a proper adjustment of pH. Fortunately, both elimination of phosphate and flocculation of microorganisms take place most efficiently within the same pH range, pH 5–6. The required alum demand for precipitation and flocculation is a function of both the concentration of dispersed microorganisms and the concentration of phosphorus present.

These considerations concerning chemical dose requirements are equally applicable to "third stage" chemical treatment of effluents of conventional biological treatment plants. Since the residual organic matter in such effluents predominantly consists of dispersed microorganisms and other "nondialyzable" components (35), final chemical cleansing by adsorption should be preceded by chemical flocculation.

Polyelectrolytes can substitute partially or fully for Al(III) in the flocculation of microorganisms. Polyelectrolytes, however, are inefficient in the elimination of phosphates. Alum and polyelectrolytes can be used jointly for phosphate removal and flocculation of microorganisms, respectively.

Flocculation at Different Phases of the Growth Rate

Since bioflocculation, as described earlier, depends on the growth phase, it appears plausible that chemical flocculability, too, is affected by the physiological conditions of the microorganisms. In order to test such an inference, some batch experiments were carried out using glucose with inorganic nutrients as a medium. In these growth experiments, aliquots were removed at various times and analyzed for concentrations of bacteria (suspended solids), glucose, and dichromate consumption (COD), and were tested for bioflocculability (percent dispersion) and flocculated with alum (chemical flocculability). Results of a representative experiment are given in Figure 12.

It first might be noted from Figure 12 that the curves describing concentrations of bacteria (Figure 12A) and glucose (Figure 12C) as functions of time are in accord with the "textbook" concepts on bacterial growth. A lag, a log growth, a declining growth phase, and an endogenous phase can be distinguished: the rate of glucose removal is independent of glucose concentration and is proportional at any time to the instantaneous concentration of microorganisms; as soon as the glucose concentration approaches zero (Figure 12C), the growth changes from a log growth to declining growth (Figure 12A).

It is significant that the use of nonspecific analytical techniques for the determination of glucose, such as COD, can lead to erroneous interpretations. A comparison of Figure 12B with 12C shows that there is a discrepancy between concentration values for COD and specifically determined glucose, especially in the range where the concentration of glucose is small and in the endogenous phase, where glucose can no longer be detected. Even after extensive periods of aeration, substantial concentrations of organic material remain in solution, as shown by COD measurements. This discrepancy is explained most easily by inferring that degradation intermediates and waste products of the cells are introduced into the solution. Some of these waste products, frequently referred to as polysaccharides, do not appear to be readily biodegradable. We have proposed earlier that some of these waste products play an important role as polymers in bioflocculation.

Figure 12D shows that, after extensive aeration into the endogenous phase, bioflocculation improved significantly. The chemical flocculability with alum, in the particular case investigated, however, does not follow the same trend as bioflocculability (Figure 12E). During the early stages

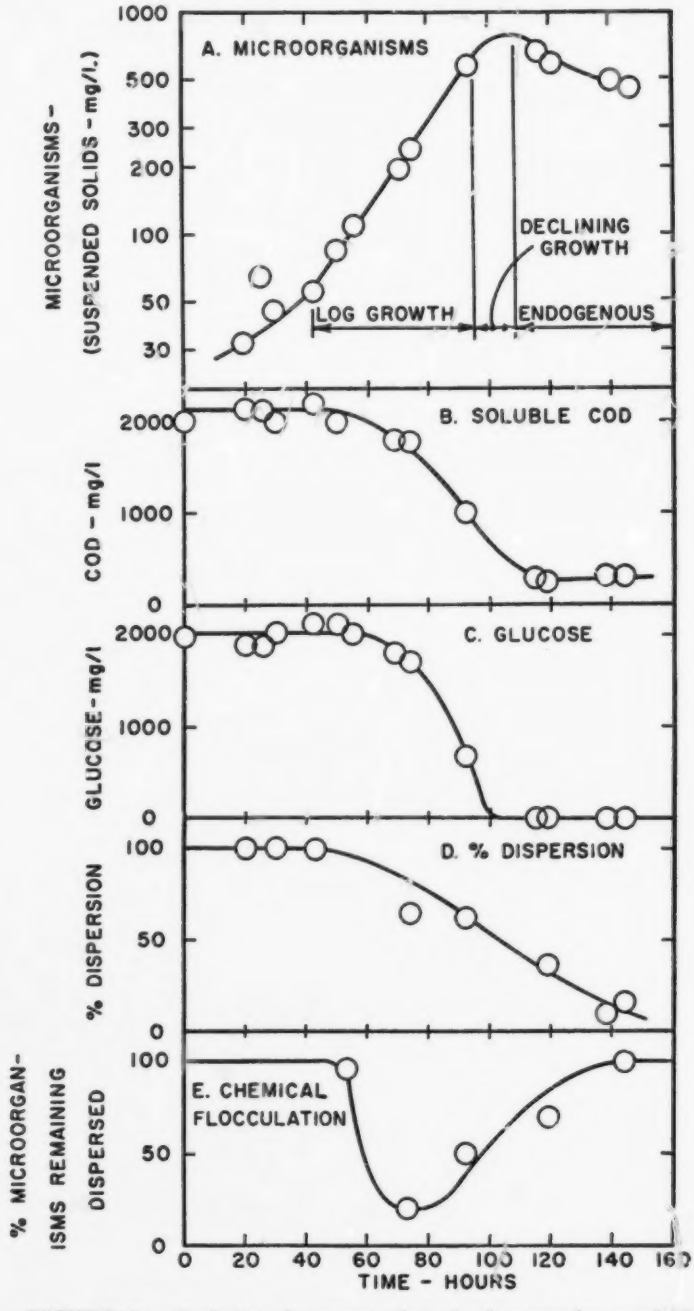

FIGURE 12.—Variation of concentration of microorganisms, soluble COD, glucose, and biological and chemical flocculability with time in batch studies.

of growth, both the biological and chemical flocculabilities are impaired. By the time the substrate has become exhausted, the chemical flocculability is at its optimum. In the endogenous phase, however, where biological self-flocculation becomes optimal, the chemical flocculation of those organisms that did not settle as a result of bioflocculation again becomes more difficult.

Microorganisms collected in this endogenous phase appear to be covered loosely with capsular and polymeric materials. If such organisms are centrifuged, this loosely bound material is sheared off easily and substantial quantities of organic material are found in the supernatant of the centrifuged samples. A redispersion of microorganisms from which such material has been sheared off can be flocculated very readily with chemical flocculants.

It should be realized that the observations reported above have been obtained with organisms growing in only one particular substrate, glucose. Our experiments with other media and with effluents of continuous culture units, however, tend to corroborate the general observation that chemical flocculation of microorganisms becomes most efficient when the concentration of soluble substrate becomes sufficiently small to limit growth (declining growth phase). It is an expedient coincidence that chemical flocculability is optimal at the particular range of the growth cycle where, in our scheme of comprehensive biological and chemical treatment of wastes, chemical flocculation treatment should be operated in order to maximize to the fullest extent the biological capabilities of the organisms and the potentialities of chemical flocculants.

Summary

The results obtained in this research demonstrate that chemical flocculation methods can complement and substitute for a part of the biological waste treatment process.

The removal of organic matter in biological treatment can be interpreted as taking place in two phases: substrate utilization, or conversion of substrate into microorganisms and oxidation of substrate; and bioflocculation, or phase separation of microorganisms from solution. Since chemical flocculation techniques can be used efficiently for the phase separation of microorganisms, the design of the biological phase can be optimized without giving consideration to the bioflocculation phase. Thus, it is possible to reduce significantly the detention time of the biological stage. Because chemical treatment can remove phosphate simultaneously, the effluents from such a complementary treatment process should, ideally, neither disturb the oxygen balance nor promote algal growth in the receiving waters.

Dispersed microorganisms behave, from a point of view of flocculation, as hydrophilic colloids. They can be flocculated readily by hydrolyzed multivalent metal ions and by synthetic organic polyelectrolytes. The various factors that influence the flocculation of microorganisms, such as physiological conditions, pH of the solution, degree of agitation, and flocculant dose, and the parameters that are pertinent in the efficient precipitation of phosphate, have been investigated. A high degree of control in the flocculation of microorganisms and the precipitation of phosphate can be achieved by a proper control of pH and degree of agitation.

A stoichiometric relation between the concentration of the chemicals necessary for flocculation and the concentration of both dispersed microorganisms and phosphate has been established. Thus, the quantity of chemical flocculants necessary can be predicted from the concentration of microorganisms and the phosphate content of the solution.

Biological self-flocculation is interpreted in terms of interaction of naturally produced polyelectrolytes which form bridges between the individual microbial particles. Chemical flocculation of microorganisms by both the hydrolysis products of polyvalent metal ions and by synthetic polyelectrolytes can be interpreted similarly in terms of such a polymer-bridge model.

Acknowledgment

This work has been supported by a research grant of the U. S. Public Health Service (WP-98) and a U. S. Public Health Service research fellowship to Mark W. Tenney (WPPM 9710-C3). The technical assistance of Mrs. Jane Shepherd gratefully is acknowledged. The kindness of many companies in supplying samples of polyelectrolytes also is appreciated.

References

1. McKinney, R. E., and Weichlein, R. G., "Isolation of Floc-Producing Bacteria from Activated Sludge." *Applied Microbiol.*, **1**, 259 (1953).
2. McKinney, R. E., and Horwood, M. P., "Fundamental Approach to the Activated Sludge Process. I. Floc-Producing Bacteria." *Sewage and Industrial Wastes*, **24**, 2, 117 (Feb. 1952).
3. McKinney, R. E., "Biological Flocculation." In "Biological Treatment of Sewage and Industrial Wastes." Vol. 1, p. 88, Reinhold Publishing Corp., New York (1956).
4. Abramson, H. A., Moyer, L. S., and Gorin, M. H., "Electrophoresis of Proteins and the Chemistry of Cell Surfaces." Reinhold Publishing Corp., New York (1942).
5. Lamanna, C., and Mallette, M. F., "Basic Bacteriology." The Williams and Wilkins Co., Baltimore, Md. (1953).
6. Stearns, T. W., and Roephe, M. H., "Electrophoresis Studies of *Brucella*." *Jour. Bacteriol.*, **42**, 411 (1941).
7. Moyer, L. S., "Changes in the Electrokinetic Potential of Bacteria at Various Phases of the Culture Cycle." *Jour. Bacteriol.*, **32**, 433 (1936).
8. Watrous, R. M., "Cataphoretic Velocity of the *Typhoid bacillus* as a Function of Culture Age and the Type of Medium." *Jour. Infect. Diseases*, **60**, 47 (1937).
9. McKinney, R. E., "Fundamental Approach to the Activated Sludge Process. II. A Proposed Theory of Floc Formation." *Sewage and Industrial Wastes*, **24**, 3, 280 (Mar. 1952).
10. La Mer, V. K., and Smellie, R. H., Jr., "Flocculation, Subsidence and Filtration of Phosphate Slimes. I. General." *Jour. Colloid Sci.* (Sweden), **11**, 704 (1956).
11. Smellie, R. H., Jr., and La Mer, V. K., "Flocculation, Subsidence and Filtration of Phosphate Slimes. VI. A Quantitative Theory of Filtration of Flocculated Suspensions." *Jour. Colloid Sci.* (Sweden), **13**, 589 (1958).
12. La Mer, V. K., and Healy, T. W., "Adsorption-Flocculation Reactions of Macromolecules at the Solid-Liquid Interface." *Rev. Pure and Applied Chem.*, **13**, 112 (1963).
13. Katchalsky, A., "Polyelectrolytes." *Endeavour*, **12**, 90 (1953).
14. Katchalsky, A., Danon, D., Nevo, A., and DeVries, A., "Interactions of Basic Polyelectrolytes with the Red Blood Cell. II. Agglutination of Red Blood Cells by Polymeric Bases." *Biochim. et Biophys. Acta*, **33**, 120 (1959).
15. Hueper, W. C., "Reactions of the Blood and Organs of Dogs after Intravenous Injections of Methyl Cellulose of Graded Molecular Weights." *Amer. Jour. Pathol.*, **20**, 737 (1944).
16. Porter, J. R., "Bacterial Chemistry and Physiology." John Wiley and Sons, Inc., New York (1946).
17. Bukantz, S. C., Cooper, A., and Bullowa, J. G. M., "The Elaboration of Soluble Capsular Polysaccharide by *Phenumococcus III* in Relation to Growth Phases in Vitro." *Jour. Bacteriol.*, **41**, 29 (1941).
18. Knaysi, G., "Elements of Bacterial Cytology." Comstock Publishing Co., Ithaca, N. Y. (1944).
19. Symons, J. M., and McKinney, R. E., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge." *Sewage and Industrial Wastes*, **30**, 7, 874 (July 1958).
20. Washington, D. R., and Symons, J. M., "Volatile Sludge Accumulations in Activated Sludge Systems." THIS JOURNAL, **34**, 8, 767 (Aug. 1963).
21. McWhorter, T. R., and Heukelekian, H., "Growth and Endogenous Phases in the Oxidation of Glucose." In "Advances in Water Pollution Research." Proc. 1st Intl. Conf. Water Pollution Research, Pergamon Press, Ltd., London, England, Vol. 2, 419 (1964).
22. Stumm, W., and Morgan, J. J., "Chemical Aspects of Coagulation." *Jour.*

17

Amer. Water Works Assn., **54**, 8, 971 (Aug. 1962).

23. Symons, J. M., McKinney, R. E., and Hassis, H. H., "A Procedure for Determination of Biological Treatability of Industrial Wastes." THIS JOURNAL, **32**, 8, 841 (Aug. 1960).
24. "Standard Methods for the Examination of Water and Wastewater." 11th Ed., Amer. Pub. Health Assn., New York (1960).
25. Engelbrecht, R. S., and McKinney, R. E., "Membrane Filter Method Applied to Activated Sludge Suspended Solids Determinations." *Sewage and Industrial Wastes*, **28**, 11, 1321 (Nov. 1956).
26. Ives, K. J., "Electrokinetic Phenomena of Planktonic Algae." *Proc. Soc. Water Trt. and Exam.*, **5**, 41 (1956).
27. Cohen, J. M., Rourke, G. A., and Woodward, R. L., "Natural and Synthetic Polyelectrolytes as Coagulant Aids." *Jour. Amer. Water Works Assn.*, **50**, 4, 463 (Apr. 1958).
28. Hodge, H. M., and Metcalfe, S. N., Jr., "Flocculation of Bacteria by Hydrophilic Colloids." *Jour. Bacteriol.*, **75**, 258 (1958).
29. Len, W. L., Rohlich, G. A., and Katz, W. J., "Removal of Phosphates from Treated Sewage." *Sewage and Industrial Wastes*, **26**, 3, 261 (Mar. 1954).
30. Sawyer, C. N., "Some New Aspects of Phosphates in Relation to Lake Fertilization." *Sewage and Industrial Wastes*, **24**, 6, 768 (June 1952).
31. Henriksen, A., "The Coagulation Process Applied to Phosphate Removal." Paper presented at the Coagulation Colloquium organized by the British Water Research Association, Feb. 1963.
32. Stumm, W., Discussion of "Methods for the Removal of Phosphorus and Nitrogen from Sewage Plant Effluents." In "Advances in Water Pollution Research." Proc. 1st Intl. Conf. on Water Pollution Research, Pergamon Press Ltd., London, England, Vol. 2, 216 (1964).
33. Gorchev, H., and Stumm, W., "The Reaction of Ferric Iron with Orthophosphate." *Jour. Inorganic Nuclear Chem.*, **25**, 567 (1963).
34. Matijević, E., and Milić, I., "On Precipitation Effects of Aluminum, Lanthanum, Iron (III), and Thorium Phosphates." *Kolloid-Zeits. und Zeits. für Polymere*, **188**, 129 (1962).
35. Bunch, R. L., Barth, E. F., Ettinger, M. B., "Organic Content of Secondary Sewage Effluents." In "Biological Treatment of Sewage and Industrial Wastes," Vol. 3 (In press).

RECEIVED
NATIONAL TECHNICAL
DEC 1 2 1972
INFORMATION SERVICE